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Searching for the competitive ability of the alien seagrass *Halophila stipulacea* with the autochthonous species *Cymodocea nodosa*

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Abstract

The tropical seagrass *Halophila stipulacea* (Forsskål) Ascherson, 1867 entered in the Mediterranean Sea through the Suez Canal more than 100 years ago. In coastal-marine ecosystems the spatial niche of *H. stipulacea* is often overlapped with that of native Mediterranean Sea seagrasses and therefore it might out-compete them. Aiming to better understand its invasiveness potential, we monitored a Southern Mediterranean shallow coastal-marine water habitat from August 2010 to August 2011, where *H. stipulacea* co-occurred with the native seagrass *Cymodocea nodosa* (Ucria) Ascherson, 1870. Besides, the year-round dynamics of *H. stipulacea* was also monitored in four periods. To test the hypothesis that the presence/absence of *H. stipulacea* may have an effect on *C. nodosa* density, we analyzed the shoot density of *C. nodosa* in 8 sites, 4 sites where *H. stipulacea* was present (impacted sites) and 4 where *H. stipulacea* was absent (control sites). The results showed significant differences in *C. nodosa* shoot density according to the presence/absence of *H. stipulacea*, with the lowest values observed in sites where it co-occurred with *H. stipulacea*. We hypothesize that the dense rhizome-sediment net created by *H. stipulacea* can interfere with *C. nodosa* density, pushing down its rhizomes in the anoxic layer. The leaf features of *H. stipulacea* were generally comparable to those of other Mediterranean populations. In January 2011 a significant decline of *H. stipulacea* was observed, maybe related to changes in the environmental conditions that have

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become unfavorable (e.g. hydrodynamics, turbidity) and, unexpectedly, the seagrass disappeared in April 2011. In January, we also observed the occurrence of the green alien alga *Caulerpa cylindracea* Sonder, 1945 which rapidly invaded the bare substrate left by *H. stipulacea*.

Keywords

Cymodocea nodosa, *Halophila stipulacea*, invasive alien species (IAS), Mediterranean Sea, non-indigenous species (NIS), seagrasses, shallow coastal-marine habitat

Introduction

Alien or non-indigenous species (NIS, i.e. organisms introduced from beyond their natural, past or present, geographical region and outside of their natural dispersal potential) are widely recognized as a major threat to native biodiversity, ecosystem functioning and services (Wallentinus and Nyberg 2007; Katsanevakis et al. 2014; Vergés et al. 2016). Besides that, they may in time become invasive (Invasive Alien Species "IAS"), determining significant environmental, socio-economic and human health impacts, such as biodiversity loss and ecosystem services degradation (Giakoumi 2014; Gallardo et al. 2016; Vergés et al. 2016). The Mediterranean Sea is a hot-spot of biodiversity (e.g. Coll et al. 2010), but the dramatically-accelerating rate of alien species introduction made it also a true hot-spot for biological invasions (Zenetos et al. 2017; Galil et al. 2018; Bariche et al. 2020; Zenetos and Galanidi 2020).

Islands, also considered hotspots of biodiversity, are vulnerable to anthropogenic pressures as well as for hosting NIS (Tershy et al. 2015; Domina et al. 2018). In particular, Sicily Island and circum-Sicilian Islands (Central Mediterranean Sea, Italy) are notably prone to biological invasions, due to their strategic position at the crossroads between the South Western and Eastern Mediterranean Sea, by virtue of the intense maritime traffic skirting the region and lessepsian migrations (Occhipinti-Ambrogi et al. 2011a, 2011b; Katsanevakis et al. 2014; Mannino et al. 2014, 2017, 2018, 2019).

Among the NIS entered in the Mediterranean Sea, there is the putative lessepsian migrant (tropical species that migrate into the Mediterranean Sea through the Suez Canal) *Halophila stipulacea* (Forsskål) Ascherson, 1867 (Hydrocharitaceae), a small seagrass native of the Red Sea, Persian Gulf, and Indian Ocean (Lipkin 1975; El Shaffai 2011). It is the only alien seagrass entered in the Mediterranean Sea given that the *Halophila* population found in Salamina Island, Greece (initially indicated as *Halophila decipiens* Ostenfeld, 1902), most likely corresponds to a morphological variant of *H. stipulacea* (Gerakaris et al. 2020; García-Escudero et al. 2022). *Halophila stipulacea*, reported for the first time in the Mediterranean Sea off the Rhodes Island coast (Fritsch 1895), spread along the Eastern Mediterranean Sea and progressively expanded westwards and northwards (see Gambi et al. 2009, 2018; Tsiamis et al. 2010; Katsanevakis 2011; Sghaier et al. 2011, 2019; Varela-Álvarez et al. 2011; Winters et al. 2020; Di Genio et al. 2021; Hoffman 2021; Pica et al. 2021; Thibaut et al. 2022), mostly invading shallow water locations close to ports and marinas, suggesting that maritime traffic was the main vector of spreading. Currently, Cannes (France) represents the most

northern location in the Western Mediterranean Sea, suggesting a rapid expansion of this species likely due to climate change and tropicalization. The species also entered in the tropical Atlantic Ocean (Caribbean Sea) where it has been rapidly expanding (Winters et al. 2020), and it is assumed that this genotype came from the Mediterranean Sea (Ruiz and Ballantine 2004; Boudouresque et al. 2016).

Halophila stipulacea is generally considered a relative fast-growing seagrass, and its success is attributed to its high morphological, physiological and biochemical plasticity and ability to spread and adapt to a wide range of environmental conditions (Gambi et al. 2009; Willette and Ambrose 2009; Mejia et al. 2016; Oscar et al. 2018; Beca-Carretero et al. 2020; Wesselmann et al. 2020; Winters et al. 2020; Thibaut et al. 2022). According to Oscar et al. (2018), the invasive nature of *H. stipulacea* in the Mediterranean and Caribbean Sea could be explained, in particular, by its remarkable tolerance to a wide range of temperatures (14–36 °C) and salinities (from 25 PSU to 60 PSU). Furthermore, Wesselmann et al. (2020) provide evidence for an upper thermal tolerance of *H. stipulacea* higher than those of the native seagrasses, particularly *Posidonia oceanica* (L.) Delile, 1813. Thus, the increase of seawater temperature in the Mediterranean Sea would improve the *H. stipulacea* performance (growth, recruitment and metabolic rates) but also its expansion into the Western Mediterranean basin (Wesselmann et al. 2020; Di Genio et al. 2021).

Moreover, Rotini et al. (2017) showed that the associated bacterial consortium may help *H. stipulacea* to better cope with environmental changes, thus having an important role in its ecological resilience and invasiveness. Additionally, its ability to acquire ammonium efficiently either from the water or from the sediment, may confer it an advantage with respect to other Mediterranean seagrasses (Alexandre et al. 2014).

As a result of its high tolerance, it has been estimated that in the near future *H. stipulacea* will be present throughout the whole Mediterranean Sea (Georgiou et al. 2016; Beca-Carretero et al. 2020; Wesselmann et al. 2020). The indigenous warm-temperate seagrass *Cymodocea nodosa* (Ucria) Ascherson, 1870, is also able to tolerate high temperatures (34–35 °C, optimum temperature at 30 °C) and a wide range of salinities (between 10 and 50 PSU) (Tsioli et al. 2019). Under climate change scenarios, it is therefore expected *C. nodosa* to cope better with environmental changes, providing it with an advantage compared with *P. oceanica*.

In the Mediterranean Sea, *H. stipulacea* frequently co-occurs with native seagrasses such as *C. nodosa*, *P. oceanica*, *Zostera noltei* Hornemann, 1832 and native or introduced macroalgae such as *Caulerpa prolifera* (Forsskål) Lamouroux, 1809, *Caulerpa cylindracea* Sonder, 1845, and *Caulerpa taxifolia* var. *distichophylla* (Sonder) Verlaque, Huisman & Procaccini, 2013 (Kashta and Pizzuto 1995; Di Martino et al. 2006; Sghaier et al. 2011; Gaglioti and Gambi 2018). Although *H. stipulacea* is listed among the worst invasive species (Boudouresque and Verlaque 2002; Streftaris and Zenetos 2006), there is little evidence of its impact on native macrophytes in the Mediterranean Sea (Tsiamis et al. 2010; Sghaier et al. 2014; Hoffman 2021; Tsirintanis et al. 2022; Conte et al. 2023). Tsirintanis et al. (2022), for instance, reported for *H. stipulacea* a medium strength of evidence of combined negative and positive impacts on biodiversity. While Hoffman (2021) stated that the invasion of *H. stipulacea* into the sub-tidal of the Levant Mediter-

ranean coast of Israel may represent a serious threat to the scarce population of *C. nodosa* down into the deep sub-tidal, as a consequence of the increased seawater temperatures. Moreover, Kolátková et al. (2021) hypothesized that a fungal parasite, recently discovered on Mediterranean *H. stipulacea*, may also affect native seagrasses such as *C. nodosa*. Recently, Conte et al. (2023) highlighted the potential capability of *H. stipulacea* to outcompete the native *C. nodosa* in the Aegean Sea, related to its morphophysiological plasticity and the ability to harbor and interact with diversified bacterial communities.

Instead, in the Caribbean Sea where it has been recently introduced, *H. stipulacea* is rapidly displacing native seagrasses (e.g. *Syringodium filiforme* Kützing, 1860) (Willette and Ambrose 2009, 2012; Willette et al. 2014; van Tussenbroek et al. 2016).

Since the ongoing tropicalization pattern of the Mediterranean Sea could facilitate *H. stipulacea* to compete with native seagrasses such as *C. nodosa* (Chefaoui et al. 2018; Savva et al. 2018; Wesselmann et al. 2020), it is mandatory to better understand the role of this alien species within native communities. Hence, we tested the hypothesis that the presence/absence of *H. stipulacea* may have an effect on native seagrasses. To do that, we surveyed from August 2010 to August 2011 a Southern Mediterranean shallow water habitat where *H. stipulacea* co-occurred with *C. nodosa*. In particular, the shoot density of *C. nodosa* in sites where *C. nodosa* co-occurred with *H. stipulacea* (impacted sites) and sites where *C. nodosa* formed monospecific populations (control sites) were analyzed. As information regarding the year-round dynamics of *H. stipulacea* in the Mediterranean Sea is scarce (Nguyen et al. 2020; Di Genio et al. 2021), we also monitored over four periods the *H. stipulacea* biometric features.

Materials and methods

Study area

The study was carried out in semi-artificial shallow water basins located in proximity to the harbour of Termini Imerese (Fig. 1; 37°59'00"N, 13°42'00"E; North-Western Sicily, Italy, Southern Mediterranean Sea). To date, this is the only location in North-Western Sicily where *H. stipulacea* records have been reported (Mannino et al. 2009). The basins (hereafter referred to as sites) range in size from 5,500 m^2 to 10,000 m^2 and are connected to the sea via a central opening. The environmental characteristics at the sites are comparable, with sandy bottoms and depths ranging from 0.8 to 2.5 meters. Salinity remained consistent (38) throughout the seasons, while temperatures ranged from 25 °C in summer to 13 °C in winter (Table 1). Preliminary surveys revealed that *H. stipulacea* is not present in all sites but becomes dominant in those nearest to the harbor, whereas *C. nodosa* increases its presence into sites further away from the harbor. However, it is not clear if the presence of *H. stipulacea* can affect the density of *C. nodosa* or if the two species can co-occur. Due to the co-occurrence of these two species in this location, as well as the similar environmental characteristics, these sites are natural mesocosms that provide a unique opportunity to investigate the interaction of *H. stipulacea* and *C. nodosa*.

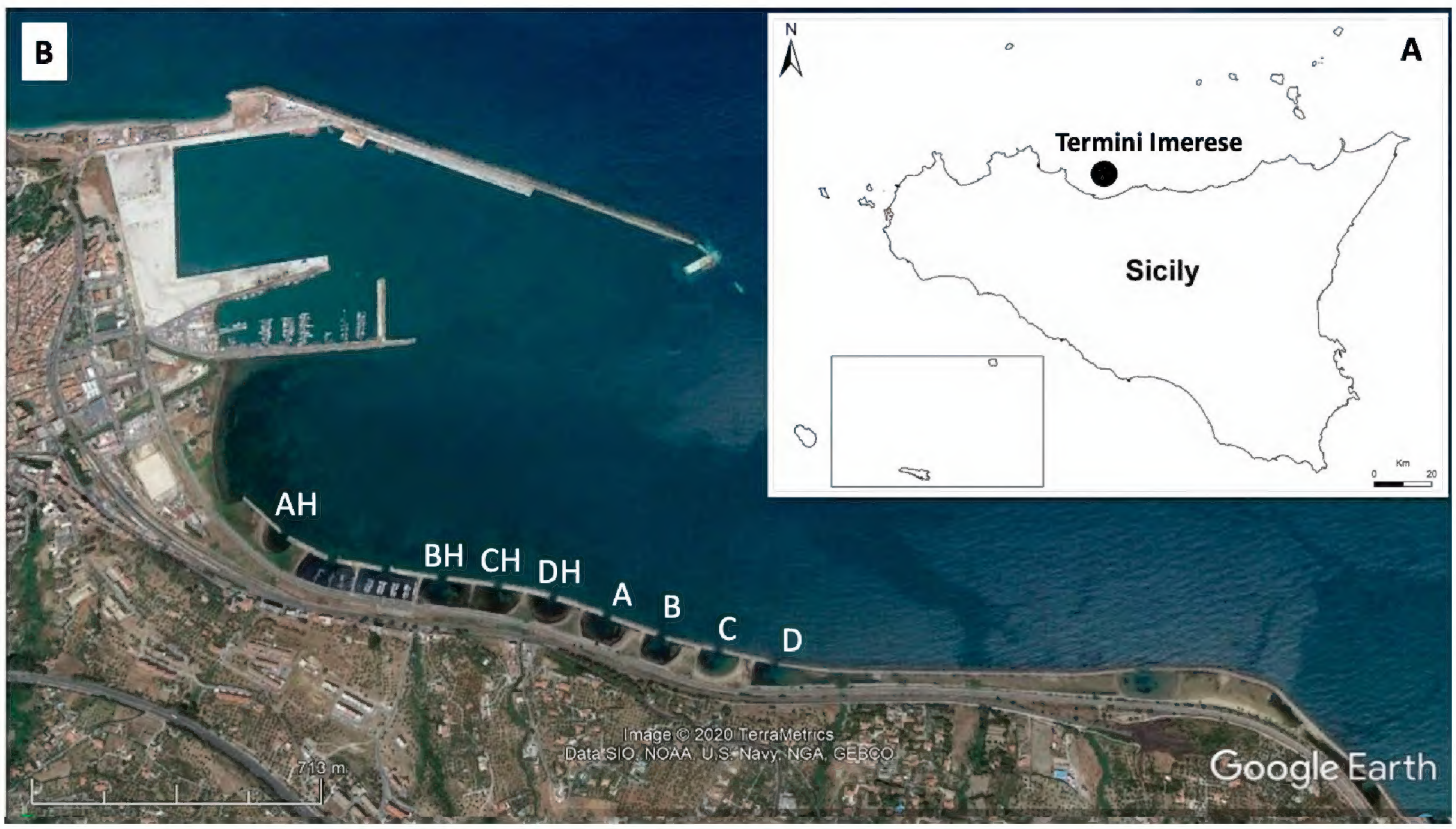

Figure 1. The study area (**A**) and details showing the investigated sampling sites (**B**). Impacted sampling sites (*Halophila stipulacea* present) are listed as: AH, BH, CH, DH. Control sampling sites (*Halophila stipulacea* absent) are listed as: A, B, C, D.

Table 1. Mean values (± SE) of measured environmental factors at the sampling sites in four periods (T1 = August, T2 = October, T3 = January, T4 = April).

Abiotic features	T1	T2	T3	T4
Temperature (°C)	24.89 ± 0.23	21.97 ± 0.16	13.97 ± 0.16	17 ± 0.12
Salinity (PSU)	38.21 ± 0.07	38.10 ± 0.06	38 ± 0.08	38 ± 0.07

Experimental design

The experiment was carried out at 8 sites. In particular, four sites (A, B, C, D; control sites) characterized by the presence of monospecific *C. nodosa* populations and four sites (AH, BH, CH, DH; impacted sites) where *C. nodosa* co-occurred with *H. stipulacea*. Sites were surveyed from August 2010 to August 2011 and samplings were carried out in four periods (T1 = August, T2 = October, T3 = January, T4 = April).

The shoot density (number of shoots/m^2) of *C. nodosa* and *H. stipulacea* was estimated by counting the number of shoots present in 3 randomly located quadrats (20 × 20 cm). The sampled shoots were brought to the lab, then were washed with seawater, sieved to remove sediment and big debris, and ultimately stored in labelled bags at 4 °C. For each site, the biometric features of *H. stipulacea* were then estimated by measuring the length and width of 30 randomly selected leaves (+/- 1 mm) in triplicate. To collect plant samples a formal permission was not required. Representative plant samples were deposited in the algological laboratory of the Department STeBiCeF - University of Palermo, Italy.

Statistical analysis

Differences in the density of *C. nodosa* among periods (fixed and orthogonal with 4 levels; T1, T2, T3 and T4), conditions (fixed and orthogonal with 2 levels; control vs impacted), sites (random and nested within conditions with 4 levels; 1, 2, 3 and 4) and their interaction were assessed using analysis of variance (ANOVA). Cochran's test was used to check for the homogeneity of variances (Underwood 1996).

To investigate which factor explained the variation in *C. nodosa* density, general mixed models (GLMs) were built using the "lme" function of the R package "nlme" (Pinheiro et al. 2016). These models included the density of *H. stipulacea*, temperature, and salinity as independent fixed continuous variables, the time as an independent random factor, and the density of *C. nodosa* as continuous dependent variable. When a variable did not follow normal distribution, it was log transformed to reach normal distribution before statistical analyses. We chose the best model for each dependent variable based on the Akaike information criterion, and we used the R package "MuMIn" (Barton 2012) to estimate variance explained by the mixed models. The number of degrees of freedom was determined using the "Kenward-Roger" method. All interactions up to the third level have been tested.

A descriptive analysis of *H. stipulacea* was carried out by calculating average (± SE) length and width of leaves, as well as its shoot density. ANOVA models were performed to investigate possible relationships between the measured variables (temperature, salinity, time, and *C. nodosa* densities) and width and length of *H. stipulacea* leaves.

Results

The *C. nodosa* shoot density was affected by the presence of *H. stipulacea*, with densities significantly lower in impacted sites compared to control ones (Fig. 2, Table 2). This effect was consistently observed through all investigated periods, although the density of *C. nodosa* tends to decrease from August 2010 to April 2011, with differences more marked in control sites than impacted ones (Fig. 2, Table 2).

Table 2. ANOVA results for testing the effects of sampling times and treatments on the density of the autochthonous species *Cymodocea nodosa*. Data were tested with the Cochran test (C=0.19; P>0.05) and then log transformed. Level of significance "P": *** <0.001; ** <0.05, * <0.1.

	DF	MS	F	P
Time	3	0.63	940	***
Treatment	1	13	19000	***
Time*Treatment	3	0.67	990	***
Treatment*Site	6	0.0014	2.1	*
Time*Treatment*Site	18	0.0014	2	**
Residuals	64	0.00068		

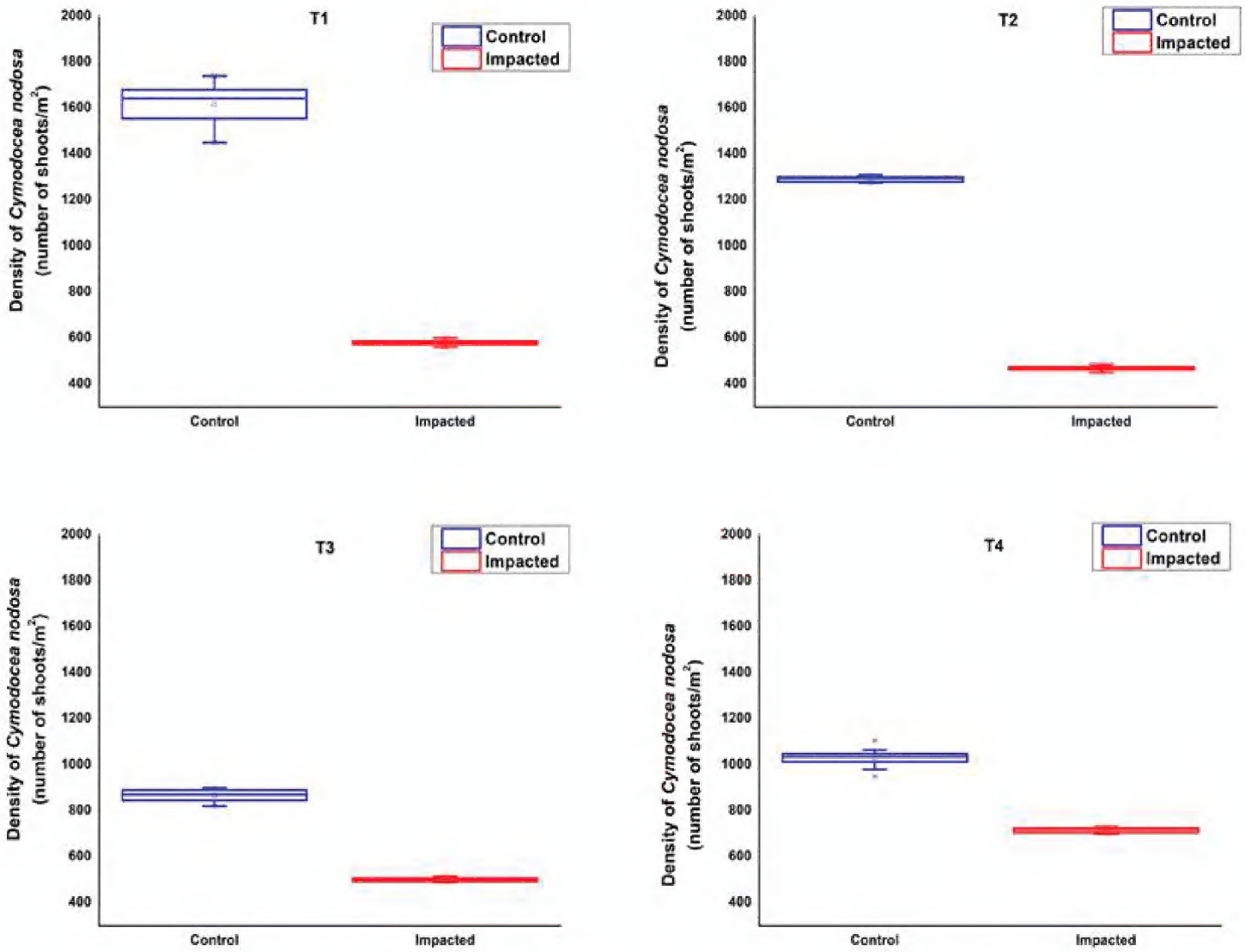

Figure 2. Variation of *C. nodosa* density in control sampling sites and in impacted sampling sites at each sampling time (T1 = August, T2 = October, T3 = January, T4 = April). As a preliminary analysis at each time, the sampling sites of each treatment were pairwise compared through a one-way ANOVA. No significant differences were found among sampling sites within the two "control" and "impacted" groups at each sampling time. The box plots were built merging the observations of each sampling site for each sampling time and treatment.

Plant interaction assessment

Shoot density of *H. stipulacea*, temperature, their interaction, as well as the interaction between *H. stipulacea* density, temperature and salinity were significant (Table 3).

At level of single predictor only the variables *H.* Density and Temperature result significant; the two variables result with negative parameters according to an inverse relationship. Consistently with the results of the ANOVA for the decomposition of the variance, the parameters of *H. stipulacea* density, temperature, their interaction and the interaction between *H. stipulacea* density, temperature and salinity were significant (Table 4).

The analysis of the first level of interaction showed that *C. nodosa* shoot density was negatively related to the increase of *H. stipulacea* density (Fig. 3A, see also Fig. 4A–C), while showing a positive relationship with temperature and salinity (Fig. 3B, C).

Table 3. Type III Analysis of Variance Table. The table reports the sum of squares, mean square, degrees of freedom of numerator and denominator, F value and Variance ratio (Pr) for each fixed independent variable and for each considered interaction (in bold significant effects). "*H.* Density" indicates the shoot density of *H. stipulacea*.

	SS	MS	NumDF	DenDF	F value	Pr(>F)
***H.* Density**	27914.595	7424.866	1.000	85.901	0.811	**0.870**
Temperature	120933.622	10872.959	1.000	87.740	1.187	**1.279**
Salinity	9856.924	9856.924	1.000	1.925	1.077	0.412
***H.* Density*Temperature**	19882.804	9149.827	1.000	85.890	0.099	**0.320**
***H.* Density*Salinity**	7957.338	7957.338	1.000	85.895	0.369	0.354
Temperature*Salinity	385561.246	385561.246	1.000	1.954	42.109	0.024
***H.* Density*Temperature*Salinity**	31951.772	14124.101	1.000	85.798	0.543	**0.718**

Table 4. ANOVA table of fixed factors. The table shows the estimated values of the fixed factors, the standard error, the degrees of freedom and the significance values for each fixed factor and for each considered interaction (in bold significant effects). "*H.* Density" indicates the shoot density of *H. stipulacea*.

	Estimate	Std. Error	DF	t value	Pr(>|t|)
(Intercept)	52631.390	24667.133	87.982	2.134	0.036
***H.* Density**	-7.227	11.250	85.813	-0.642	**0.722**
Temperature	-2195.345	1397.647	87.558	-0.571	**0.620**
Salinity	-1389.506	646.783	87.994	-2.148	0.034
***H.* Density*Temperature**	0.257	0.482	85.982	0.534	**0.595**
***H.* Density*Salinity**	0.183	0.296	85.811	0.618	0.538
Temperature*Salinity	59.563	36.622	87.629	1.626	0.107
***H.* Density*Temperature*Salinity**	-0.007	0.013	85.977	-0.517	**0.606**

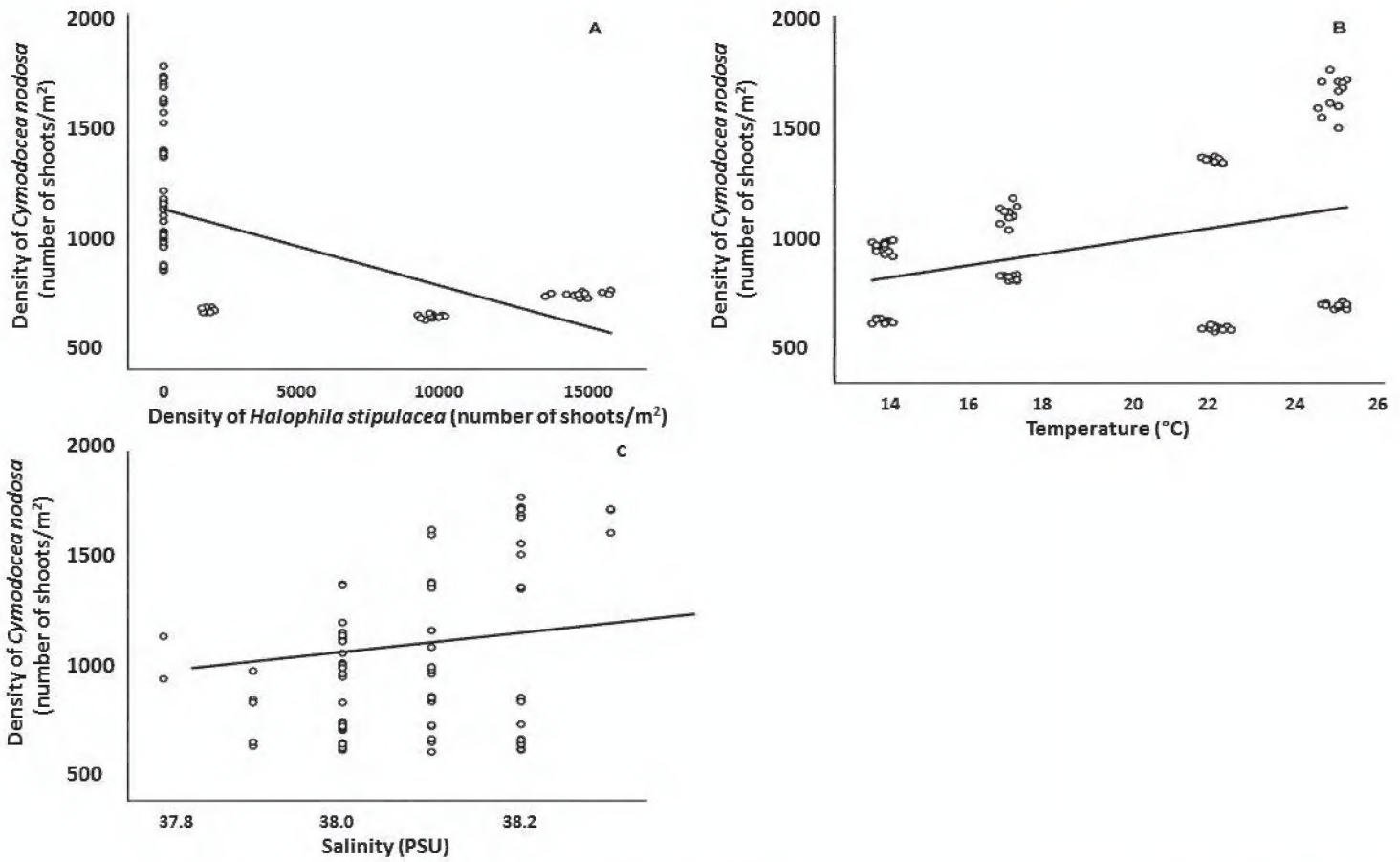

Figure 3. Relationship between *C. nodosa* density and *H. stipulacea* density (**A**), temperature (**B**) and salinity (**C**).

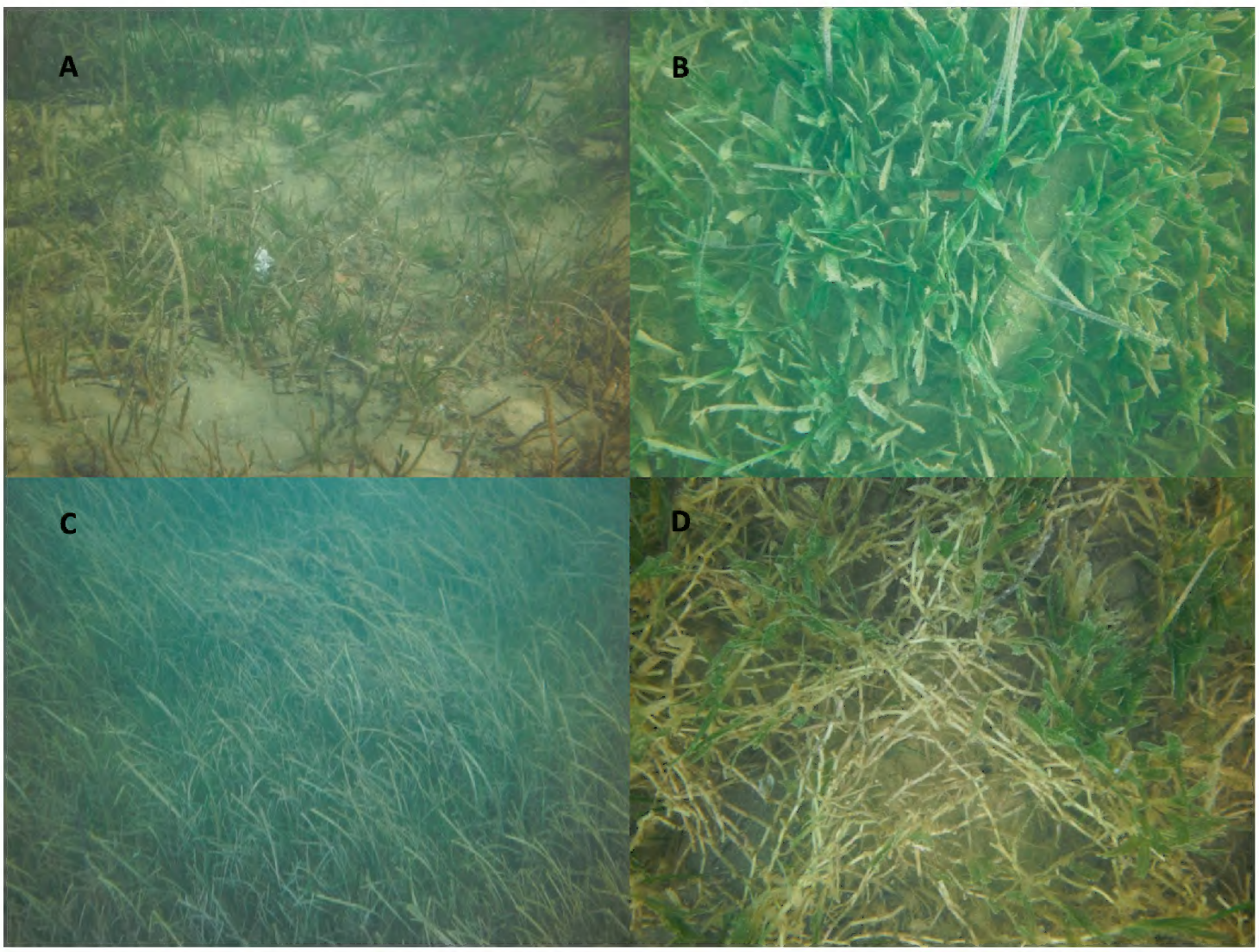

Figure 4. Examples of habitat structure at the investigated sites. *Cymodocea nodosa* in presence of *Halophila stipulacea* (**A**), *Halophila stipulacea* dominating *Cymodocea nodosa* (**B**), *Cymodocea nodosa* in absence of *Halophila stipulacea* (**C**), multi-layered mat formed by rhizomes of *Halophila stipulacea* (**D**).

We observed a dense multi-layered mat formed by the lateral rhizomes of *H. stipulacea*, growing between *C. nodosa* shoots and entrapping sediment (Fig. 4D). Therefore, in control sites rhizomes of *C. nodosa* grew above the sediment or were slightly covered by it (Fig. 5A) while in impacted sites they were pushed down in the anoxic layer by the multi-layered mat (Fig. 5B).

The second level interactions, on the other hand, produced negative relationships for the interaction between *H. stipulacea* density and temperature (Fig. 6A), and between *H. stipulacea* density and salinity (Fig. 6B); while the interaction between temperature and salinity showed a positive relationship with the density of *C. nodosa* (Fig. 6C). The third level interaction shows negative relationships for all the considered conditions (Fig. 6D).

The results of the mixed model clearly showed 3 different effects of the considered independent variables on *C. nodosa* density values. Temperature had a positive effect, *H. stipulacea* density had a negative effect and salinity (in the recorded range of values) showed a conservative effect. Their interactions clearly showed the strength of the interaction with the presence of *H. stipulacea*, a relationship appearing limited by the temperature that functions as a control variable on the negative effect led by the density of *H. stipulacea* (Fig. 6A, D).

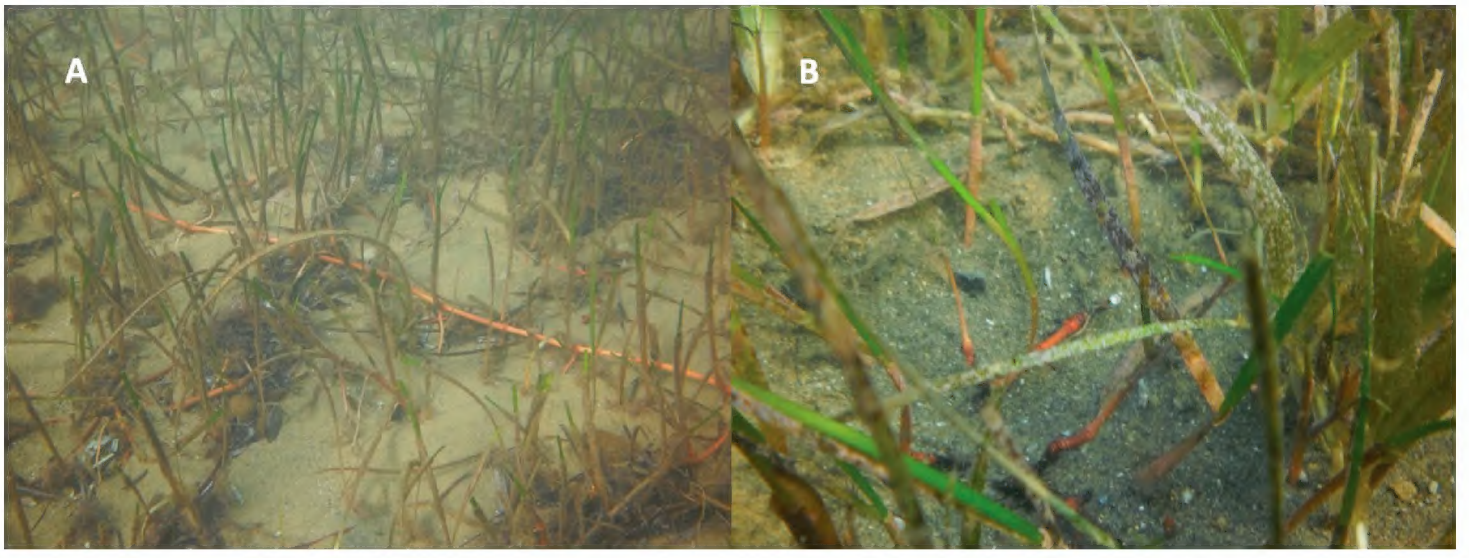

Figure 5. *Cymodocea nodosa*: rhizomes above the sediment (**A**), rhizomes pushed down in the anoxic layer (**B**).

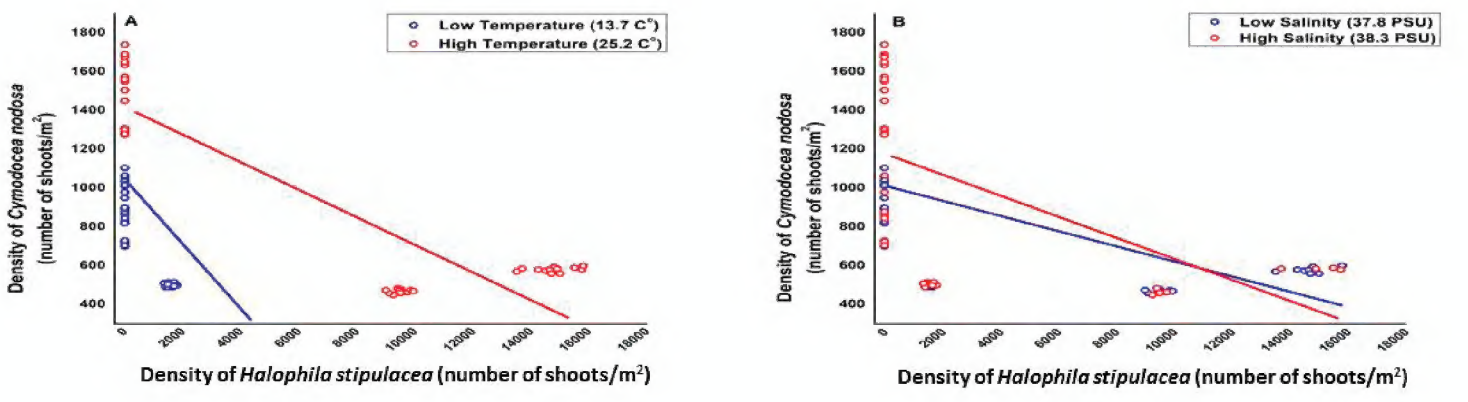
A
Low Temperature (13.7 C°)
High Temperature (25.2 C°)
Density of Cymodocea nodosa (number of shoots/m²)
Density of Halophila stipulacea (number of shoots/m²)
B
Low Salinity (37.8 PSU)
High Salinity (38.3 PSU)
Density of Cymodocea nodosa (number of shoots/m²)
Density of Halophila stipulacea (number of shoots/m²)
C
Low Salinity (37.8 PSU)
High Salinity (38.3 PSU)
Density of Cymodocea nodosa (number of shoots/m²)
Temperature (°C)
D
Low Temperature (13.7 C°)
High Temperature (25.2 C°)
Low Salinity (37.8 PSU)
High Salinity (38.3 PSU)
Density of Cymodocea nodosa (number of shoots/m²)
Density of Halophila stipulacea (number of shoots/m²)

Figure 6. Plot of the second (**A–C**) and third (**D**) level interactions, the figure shows the plot of the relationship resulting from the interaction between *H. stipulacea* density and temperature (**A**), *H. stipulacea* density and salinity (**B**), temperature and salinity (**C**) and for the interaction between all the fixed factors (**D**).

Biometric features of *H. stipulacea*

Biometric features of *H. stipulacea* are reported in Fig. 7A, B. Elliptic leaf blades, typically characterized by a distinct mid vein with many branched cross veins, were long on average 59.0 mm ± 1.8 (specifically, 59.1 mm ± 1.6 in T1, 58.8 mm ± 1.8 in T2 and 59.2 mm ± 1.8 in T3) and wide on average 6.8 mm ± 0.175 (6.921 mm ± 0.15 in T1, 6.8 mm ± 0.16 in T2 and 6.7 mm ± 0.20 in T3; Table 5). Leaf length and width didn't show significant differences among sites and sampling periods ($P>0.05$; Fig. 7A, B). Shoot density of *H. stipulacea* showed a mean value of 8,613.33 ± 384.31 number of shoots/m^2 and a consistent reduction from T1 to T3

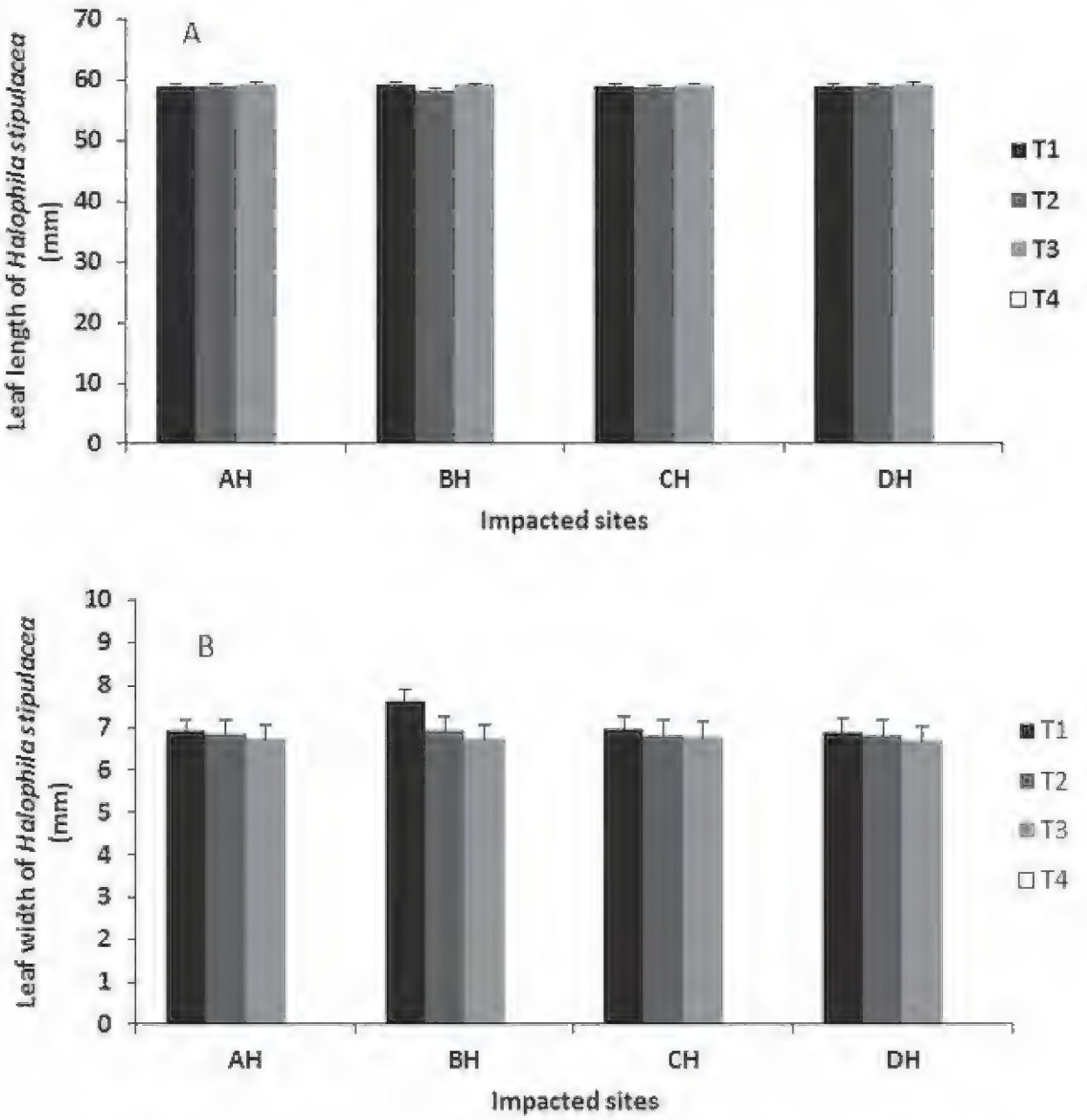

Figure 7. Leaf length (**A**) and leaf width (**B**) of *H. stipulacea* in impacted sampling sites. Bars show mean ± SE (n = 30). In T4 the species disappeared.

was observed (14,754.17 ± 722.51 in T1, 9,495.83 ± 287.98 in T2, and 1,590.0 ± 142.45 in T3). In T4 *H. stipulacea* totally disappeared except for the occurrence of a few dead shoots. Flowers have never been observed. Notably, we observed the occurrence in T3 of *C. cylindracea* (cover of 2.5%), that rapidly invaded the bare substrate left by *H. stipulacea*, reaching a cover of 40% in T4. Surveys carried out in August 2011 confirmed the presence of *C. cylindracea*, which reached a cover of about 70%.

As the best three-way ANOVA model for the "leaf width" variable, the model composed by the variables time, temperature and *C. nodosa* density was selected, based on the values of R^2 (0.993) the model explains 99% of the observed variability. The best three-way ANOVA model built for the "leaf length" variable is instead the model composed of the categorical variable "Time", based on the values of R^2 (0.931) the model explains 93% of the observed variability. In both cases based on the values of the Fisher statistic (F), the information brought by the explanatory variables is significantly better than a basic mean would bring (Table 6).

For each selected model the interactions up to the third level were evaluated; in both models the only highly significant variable is time, the variables temperature, salinity and *C. nodosa* density are weakly significant (Table 7). The interactions temperature*time, salinity*time, *C. nodosa* density*time and *H. stipulacea* density*time are weakly significant (Table 7); while all other variables and other interactions are not significant.

Table 5. Biometric data (mean ± SE) of *H. stipulacea* in the Mediterranean Sea.

Localities	References	Depth (m)	Mean density (No. of shoots/m²)	Mean leaf length (mm)	Mean leaf width (mm)
Termini Imerese harbor (Italy)	Present study	0.8 – 2.5	8,613.3 ± 384.31	59.07 ± 1.80	6.83 ± 0.17
Palinuro harbor (Italy)	Gambi et al. (2009) Di Genio et al. (2021)	2 – 5 1.8 – 4	10,500 ± 2,700 from 6,100 ± 953.9 to 9,290 ± 2,482	33.3 – 55.7 25.0–50.0	4.4 – 6.8 4.5 – 7.0
Peninsula of Maddalena (Italy)	Di Martino et al. (2006)	21	1,967	42 – 73	m.d.
Vulcano Island (Italy)	Procaccini et al. (1999)	5 – 25	12,795 – 15,170	40.3 – 67.5	5.1 – 7.8
Oliveri-Tindari (Italy)	Procaccini et al. (1999)	2	25,345 ± 4,324	63.8 – 84.3	8.3 – 10.1
Naxos-Taormina (Italy)	Cancemi et al. (1994)	2	19,728	m.d.	m.d.
Marina Cap Monastir (Tunisia)	Sghaier et al. (2011)	1 – 2	9,900 ± 3,509	58.2 ± 4.3	7.1 ± 0.7
Tobrouk Bay (Libya)	Sghaier et al. (2011)	1 – 1.5	476 ± 83	47	55
Cannes (France)	Thibaut et al. (2022)	11 – 17	202	up to 57	m.d.

m.d. = missing data.

Table 6. ANOVA table of the two computed three-way ANOVA models. WModel and LModel indicate the model constructed for the variable "*H. stipulacea* leaf width" and "*H. stipulacea* leaf length" respectively.

Source	DF	SS	MS	F	Pr > F	P
WModel	13.000	428.876	32.990	354.997	**<0.0001**	***
Error	34.000	3.160	0.093			
Corrected Total	47.000	432.036				
LModel	7.000	31405.311	4486.473	88002.034	**<0.0001**	***
Error	40.000	2.039	0.051			
Corrected Total	47.000	31407.351				

Level of significance codes (P): 0 < *** < 0.001.

Discussion

Although *H. stipulacea* is listed among one of the worst invasive species (Streftaris and Zenetos 2006), there is no clear evidence of competition with Mediterranean seagrasses, possibly due to its small shoot size compared to that of larger native species (Apostolaki et al. 2019). The co-occurrence of *H. stipulacea* and *C. nodosa* has been already reported for the Mediterranean Sea but negative interactions between the two seagrasses were not clearly observed except for the Tunisian and Crete (Greece) coasts (Kashta and Pizzuto 1995; Di Martino et al. 2006; Sghaier et al. 2014; Conte et al. 2023).

Table 7. Type III Analysis of Variance. The table reports the values of degrees of freedom, sum of squares, mean squares, F statistic and P-value for the factors and the interactions which resulted at least scarcely significant (0.1 < * < 1). Factors and interactions not present were found to be insignificant. Significant interactions are reported.

Source	DF	SS	MS	F	Pr > F	P	DF	SS	MS	F	Pr > F	P
	leaf length						leaf width					
Temperature	1.000	0.009	0.009	0.145	0.706	*	1.000	0.030	0.030	0.324	0.573	*
Salinity	1.000	0.037	0.037	0.630	0.433	*	1.000	0.069	0.069	0.748	0.393	*
C. nodosa Shoot density	1.000	0.019	0.019	0.316	0.578	*	1.000	0.006	0.006	0.066	0.798	*
Time	3.000	0.905	0.302	5.122	**0.005**	**	3.000	0.334	0.111	0.041	**0.324**	**
Temperature*Time	3.000	0.023	0.008	0.132	0.940	*	3.000	0.476	0.159	1.713	0.183	*
H. stipulacea density*Time	3.000	0.962	0.321	5.444	**0.004**	**	3.000	0.258	0.086	0.931	0.437	*
C. nodosa density *Time	1.000	0.017	0.017	0.282	0.599	*	1.000	-0.338	-0.338	-3.655	1.000	*

Level of significance codes (P): 0.001 < ** < 0.01, 0.1 < * < 1.

Sghaier et al. (2014) reported a relevant competition between *C. nodosa* and *H. stipulacea* in Tunisian coast, with *H. stipulacea* displacing till 50% of *C. nodosa*. In Crete, Conte et al. (2023) highlighted severe signs of stress (altered associated bacterial communities, biometric and biochemical descriptors) in *C. nodosa* due to the proximity of *H. stipulacea*. In the Caribbean Sea, instead, a significant competition for space was reported between *H. stipulacea* and the native seagrass *S. filiforme*, with *H. stipulacea* rapidly expanding and displacing the local seagrass but also altering the seagrass community (Willette and Ambrose 2012).

The observed values of shoot density and the total absence of flowers and/or fruits in *C. nodosa* in impacted sites might be linked to a negative effect of *H. stipulacea* on *C. nodosa* growth. The system outlined by the physical conditions (temperature and salinity) and the presence of *H. stipulacea*, analyzed through a mixed model approach, showed different effects on the density of *C. nodosa*. The temperature is the main favoring factor with respect to the density of *C. nodosa* while the density of *H. stipulacea* has a strongly limiting effect on the density of *C. nodosa*. A positive correlation between temperature and shoot density, phenological parameters (number of leaves, leaf length, leaf width and biomass) and leaf elongation rates of *C. nodosa* has been found by other authors (Pérez and Romero 1992; Marbà et al. 1996; Cancemi et al. 2002; Tsioli et al. 2019). As already observed in other Mediterranean coasts, *C. nodosa* shows strong seasonal patterns in shoot density and phenological parameters, reaching the highest values in summer (Pérez and Romero 1992; Cancemi et al. 2002; Tsioli et al. 2019). The interactions showed that the strongest relationship is the one with *H. stipulacea*; in fact all interactions with it (*H. stipulacea* density-temperature, *H. stipulacea* density-salinity and third level interaction) are all negative. The temperature seems to have a buffering effect on the negative effect of *H. stipulacea* density, in particular the limiting effect is much stronger in high temperature conditions. We hypothesized that the limiting effect is due to the dense multi-layered mat formed by *H. stipulacea* rhizomes plus sediment, which push down *C. nodosa* rhizomes in a layer with oxygen depletion

(van Tussenbroek et al. 2016). Higher temperatures will cause an increase in oxygen depletion. The behavior of *H. stipulacea* seems comparable to that of invasive *Caulerpa* spp., able to negatively affect seagrass growth through the modification and deterioration of sediments (Borum et al. 2004; Holmer et al. 2009). According to Ceccherelli and Campo (2002), *C. cylindracea* would affect *C. nodosa* shoot density, but no clear evidence of a negative interaction between these two macrophytes was found.

The leaf features of the studied *H. stipulacea* population were generally comparable to those of other Mediterranean populations (Table 5). Moreover, we observed that the dimensions of the leaves of *H. stipulacea* do not seem to strictly depend either on the measured environmental variables or on the densities of the two species. In fact, they seem to depend on the categorical variable "Time", reflecting a temporal trend. On the other hand, shoot density of *H. stipulacea* presented some differences with respect to other Mediterranean populations (Table 5). The decline of *H. stipulacea* in January 2011 and the subsequent disappearance in April, could be related to changes in the environmental conditions that have become unfavorable (e.g. hydrodynamics, turbidity). Moreover, the occurrence in January of *C. cylindracea*, which rapidly expanded, invading the bare substrate left by *H. stipulacea*, may have prevented the re-establishment of *H. stipulacea*. Negative interactions and competitiveness between *Caulerpa* sp. and *H. stipulacea* have been already observed (Di Martino et al. 2006; Gab-Alla 2007).

Fertile plants of *H. stipulacea* were not observed in the study area. We know that they are much less common in the Mediterranean Sea than in the native habitat, suggesting a difficulty in completing sexual reproduction under the Mediterranean environmental conditions. Male flowers were mainly recorded in the Western Mediterranean (Cancemi et al. 1994; Procaccini et al. 1999; Gambi et al. 2009, 2018; Di Genio et al. 2021), suggesting that female flowers are not able to develop under the Western Mediterranean environmental conditions (Gambi et al. 2009). The recent finding of fertile plants bearing fruits on Chios Island and on Turkey coasts (Gerakaris and Tsiamis 2015; Dural et al. 2020), and flowers in Cyprus (Nguyen et al. 2018), indicates that in the Eastern Mediterranean *H. stipulacea* is able to reproduce sexually. Instead, the recent record of fruits in the Caribbean Sea (Chiquillo et al. 2019) has been questioned by Smulders et al. (2020), who believe that they likely are male flower buds, which have similar dimensions to fruits. The authors stated that the existence of female flowers and fruits of *H. stipulacea* in the Caribbean Sea is still an open question and confirmed that *H. stipulacea* reproduces only asexually in the Caribbean.

Since sexual reproduction has rarely been reported in invaded areas (Mediterranean and Caribbean Sea), the dominant way of dissemination and expansion seems to be vegetative propagation (Boudouresque and Verlaque 2002; Missaoui et al. 2003; Sghaier et al. 2011; Nguyen et al. 2018). Understanding reproduction of *H. stipulacea* in its invasive range is critical to managing this species. Indeed, sexual reproduction may increase the dispersal capacity and the adaptive capacity of this species and, through the seed banks, may increase its resilience to disturbances (Ackerman 2006; Unsworth et al. 2015; Smulders et al. 2020).

Although *H. stipulacea* highlights a relatively limited invasion success in the Mediterranean Sea if compared with the successful invasion reported for the Caribbean (see Winters et al. 2020) and has never shown any clear and regular invasive behavior (Di Martino et al. 2006; Gambi et al. 2009; Sghaier et al. 2011), it shows the potential for long distance dispersal (Willette and Ambrose 2009; Short et al. 2010) and possesses some features that could make it a potential threat to native seagrasses. Under warming scenarios, *H. stipulacea* could occupy the niche left by *P. oceanica* together with *C. nodosa*, continuing to support carbon sequestration, thus contributing in the mitigation of the global warming (Wesselmann et al. 2021). However, even though *H. stipulacea* might potentially contribute to increasing the carbon sequestration, the mainly allochthonous origin of organic carbon deposited in *H. stipulacea* sediments make it more susceptible to remineralization, implying a deterioration in the quality and quantity of the carbon. Furthermore, the weak rhizome structure of the species enhances the probability of sediment erosion and subsequent loss of sedimentary carbon stock (Apostolaki et al. 2019).

Conclusion

Certainly, our results represent a starting point and further investigation on the ecology and dynamics of *H. stipulacea* and its interaction with native seagrasses is needed. Indeed, in recent years, seagrass ecosystems have been experiencing a well-documented decline in many areas of the world (Boudouresque et al. 2009; Marbà and Duarte 2010; Marbà et al. 2014; Chefaoui et al. 2018), thus their conservation is becoming increasingly important (Unsworth et al. 2019). Under the climate change scenarios, the exotic *H. stipulacea* and the native warm tolerant species *C. nodosa* could substitute *P. oceanica* or even *H. stipulacea* could significantly outcompete the *Cymodocea* native species (Wesselmann et al. 2020). Genomic can be an important tool to better understand the ability of *H. stipulacea* to adapt to environmental conditions and spreading, and to respond to expected climate change (Specchia et al. 2017; Tsakogiannis et al. 2020; Winters et al. 2020; Zangaro et al. 2021).

CRediT author statement

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